The Journal
of Research
on the Lepidoptera

Volume 44: 29-33

THE LEPIDOPTERA RESEARCH FOUNDATION, 6 June 2011

ISSN 0022-4324 (print)
ISSN 2156-5457 (online)

Note

Nutritional ecology of the mimetic butterfly *Hypolimnas missipus* L. (Lepidoptera: Nymphalidae) in Ghana

Basic information needed for conservation of insect species, especially butterflies and moths, includes larval host plants for various regions. Surprisingly, the identities of even the major larval food plants for many butterflies and moths remain unknown, particularly in the tropics.

One of the most common butterflies in agro ecosystems in Africa is *Hypolimnas missipus* Linnaeus 1764 (Lepidoptera: Nymphalidae) (Owen, 1971). This species is one of the best-studied members of the genus *Hypolimnas* in terms of its distribution, polymorphism, genetics, mimicry and biochemistry (Owen, 1971; Smith, 1976; Vane-Wright *et al.*, 1977; Gordon & Smith, 1989). Food plants reported for *H. missipus* represent at least seven plant families: Convolvulaceae, Malvaceae, Acanthaceae, Amaranthaceae, Portulacaceae, Moraceae, and Palmae (Vane-Wright *et al.*, 1977). With such a broad range of food plants, it is possible that *H. missipus* shows geographical or local adaptation to particular food plants, or that cryptic species are involved.

In this study, larval food plants of *H. missipus* in the Cape Coast area of the coastal zone of Ghana were identified and the performance of the butterfly on each plant was assessed. The nutritional contents of the food plants were analyzed to assess their possible effects on larval growth and development. Field and laboratory studies were carried out to learn which plants were used as oviposition sites by *H. missipus*. It is expected that the results will contribute to the knowledge of the specific resource needs of *H. missipus* and such knowledge will enable better management of the habitat features that help maintain its populations. There are some indications that local populations are declining, though this is yet to be quantified.

Received: 8 December 2010
Accepted: 1 January 2011

Field studies were carried out from June 2009 to March 2010 in Cape Coast in the coastal zone of Ghana. The area has double rainfall maxima totaling between 750 mm and 1000 mm per year, with the major rainy season between April and July and the minor rainy season between September and November. The mean monthly relative humidity varies between 85% and 99%. The vegetation in the metropolis consists of shrubs about 1.5 m high, grasses, and remnant forest fragments or thickets.

Observations of daily activities of *H. missipus* were carried out in and around the Research Farm (05° 07.926'N, 001° 17.588'W) and Botanical Garden (05° 06. 985'N, 001° 17.744'W) of the University of Cape Coast, and in backyard gardens and lawns of private houses in Cape Coast (05° 06. 567'N, 001° 17.294'W). Records were made of the species of plants on which adult butterflies fed or laid their eggs. Plant species that were already known as larval food plants, from previous studies, were searched for *H. missipus* larvae. Ovipositing females were observed in the field for other plants that served as oviposition sites.

Based on the field observations, *Portulaca oleracea* Linnaeus 1753, *Portulaca quadrifida* Linnaeus 1767, *Asystasia gangetica* (L) T. Anderson 1860 (Acanthaceae), *Acanthus* sp. (Acanthaceae) and *Axonopus compressus* (S.W.) P. Beauv 1812 (Poaceae) were selected to test in the laboratory, their suitability as substrates for oviposition. Female *H. missipus* (assumed already mated) were caught in the field with an aerial net and taken to the laboratory. Each butterfly was placed in a plastic tray containing one of the selected plants. A mixture of *P. quadrifida* and each of the other plants was also set up in a separate tray and a female butterfly placed in the tray. Each tray was covered with a nylon mesh and placed under an incandescent bulb, during the day, to provide light and warmth. Each set-up had 5 replicates. The butterflies were fed on dilute honey solution. The plants were observed for eggs each day for five days.

The common plants in the study area that were known as food plants of *H. missipus* (Vane-Wright *et al.*, 1977) were *P. oleracea*, *P. quadrifida*, *P. foliosa* Ker. Gawl. 1824, *P. grandiflora* Hooker 1829, *Talinum triangulare* (Jacq.) Willd 1799, (Portulacaceae) and *Asystasia*

gangetica (Acanthaceae). *Acanthus sp.* (Acanthaceae) and *Axonopus compressus* (Poaceae) were not known as food plants but were included in this study because female *H. missipus* had been observed flying about them and, on one occasion in Cape Coast, an egg had been found on each of these plants. Larvae were introduced on these plants and their growth monitored until they pupated. Each set-up consisted of a specific food plant and a single neonate larva placed in a plastic cup covered with mesh. The set-up for each food plant was replicated 10 times.

Based on our preliminary study, *P. oleracea*, *P. quadrifida* and *A. gangetica* were selected for more detailed study. Neonate larvae (from eggs laid on *P. quadrifida*) were introduced on these plants, soon after hatching and before feeding began, and their growth and development were monitored until pupation. Each set-up consisted of a specific food plant and a single neonate larva placed in a plastic cup covered with fine mesh. The set-up for each plant was replicated 50 times. The study was carried out in a laboratory with a constant temperature of 28°C and a relative humidity of 70–85%. The number of molts and the durations of larval and pupal periods were recorded. The lengths of the larvae (at hatching and before pupation) and the wing spans and body lengths of the adults were measured. Weights of day-old larvae, 4th or 5th instars (a day before pupation), and pupae (a day before emergence) were also recorded.

To determine the nutrient contents of food plants, the moisture, crude protein, crude fat, fiber, ash, and soluble carbohydrate levels of *P. oleracea*, *P. quadrifida* and *A. gangetica* were measured as described below.

Samples of each food plant were weighed and dried in an oven at 105°C until constant weights were reached. Moisture content was calculated as

$$\text{Moisture (\%)} = \frac{\text{loss in weight on drying (g)}}{\text{initial sample weight (g)}} \times 100$$

Crucibles were pre-heated in a muffle furnace to about 500°C then cooled in a dessicator and weighed. Crucibles containing 1 g of dry matter of each sample were placed in a cold muffle furnace. The temperature was allowed to rise to 500°C and after 3 hours at 500°C, the crucible was removed, allowed to cool and weighed to determine ash content.

$$\text{Ash (\%)} = \frac{\text{ash weight (g)}}{\text{oven dry weight (g)}} \times 100$$

Total organic nitrogen (N) was determined in the samples by the Kjeldahl digestion and steam distillation procedure as described by Stewart *et al.* (1974).

$$\text{Crude protein (\%)} = \text{N (\%)} \times 6.25$$

In determining the crude fat, 1 g of dry matter from each sample was weighed, placed into a 50 x 10

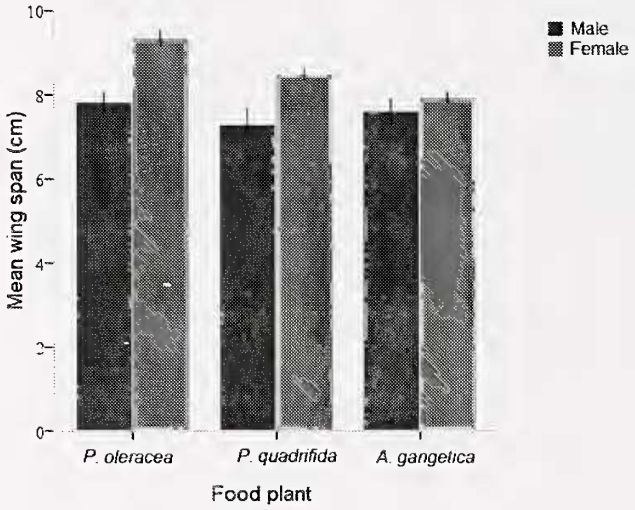

Figure 1. Wing spans of adult male and female *H. missipus* reared from larvae that developed on *P. oleracea*, *P. quadrifida* and *A. gangetica*.

Figure 2. Body lenghts of adult male and female *H. missipus* reared from larvae that developed on *P. oleracea*, *P. quadrifida* and *A. gangetica*.

mm Soxhlet extraction thimble, and then transferred to a 6 ml capacity Soxhlet extractor. About 20 ml of ether was added to a 25 ml round-bottomed flask (B14) containing a glass bead. It was connected to the extractor and extracted for 4 to 6 hours using a heating mantle. The flask was removed and placed in a warm water bath, where the ether was evaporated off using a steam of oxygen-free N_2. It was then placed in a vacuum oven at 40°C for 30 min after which it was cooled in a desiccator and re-weighed.

$$\text{Crude fat (\%)} = \frac{\text{residue in ether extract (g)} \times 10^2}{\text{sample weight (g)}}$$

Soluble carbohydrates were determined in the samples using hot water extraction as described by Stewart *et al.* (1974). For crude fiber content, samples were boiled successively with 1.25% w/v sulphuric acid and 1.25% w/v sodium hydroxide as described by Stewart *et al.* (1974).

The data were tested to verify normality (Shapiro-Wilks test) and the homogeneity of variances. A nonparametric test was used to analyse data that were not normally distributed. Thus the Mann-Whitney and Wilcoxon tests and Kruskal-Wallis test were carried out, as well as multiple comparisons by ranks. The significance of differences between males and females were determined by the Mann-Whitney and Wilcoxon tests, and the Kruskal-Wallis test was used to analyze the growth performances among larvae reared on the three food plants. All statistics were performed by the use of SPSS (version 16) application software.

Adult *H. missipus* were seen feeding on flowers of *Tridax procumbens* L. 1753, *Talinum triangulare* (Jacq.)

Table 1. Development periods of *H. missipus* on three food plants.

Food plant	Larval period (days) †		Pupal period (days) †		Larva-adult (days) †	
	male	female	male	female	male	female
P. oleracea	13.2^{1a}	13.8^{2a}	9.4^{1a}	9.9^{2a}	22.5^{1a}	23.7^{2a}
P. quadrifida	16.7^{1b}	17.4^{2b}	9.1^{1a}	9.6^{1a}	25.8^{1b}	26.9^{2b}
A. gangetica	22.8^{1c}	25^{2c}	8.9^{1a}	9.9^{2a}	31.7^{1c}	34.9^{2c}

† Values in columns not sharing the same letters or in rows within a period not sharing the same numbers are significantly different at the 5% level.

Table 2. Duration of *H. missipus stadia* on three food plants.

Food plant	Period of larval growth (days)				
	1st instar	2nd instar	3rd instar	4th instar	5th instar
P. oleracea	4.5	2.5	1.5	4.5	-
P. quadrifida	6	2.5	2.5	6	-
A. gangetica	4.5	2.5	2.5	6	7

Table 3. Mean weights (± SE) of pupae that developed on three food plants.

Food plant	Mean pupal weight (g) †	
	Male	Female
P. oleracea	$0.77^{1a} \pm 0.02$	$0.95^{2a} \pm 0.01$
P. quadrifida	$0.63^{1b} \pm 0.02$	$0.77^{2b} \pm 0.03$
A. gangetica	$0.64^{1b} \pm 0.01$	$0.73^{2b} \pm 0.02$

† Values in columns not sharing the same letters or in rows not sharing the same numbers are significantly different at 5% level.

Willd.1799, *Melanthera scandens* (Schumach.) Roberty 1954 and *Lantana camara* L. 1753. In the field, the females laid clutches of eggs on *P. oleracea* and *P. quadrifida*. However, in some instances, single eggs were laid on *Acanthus* sp. and *Axonopus compressus*. Larvae were found eating leaves of *P. oleracea* and *P. quadrifida* in the field.

In the laboratory, eggs were laid only on *P. quadrifida* when plants were provided separately. However, eggs were also laid on *P. oleracea*, *Asystasia gangetica* and *Axonopus compressus* when mixed with *P. quadrifida*. Larvae survived on *P. oleracea*, *P. quadrifida* and *A. gangetica* but none of the larvae survived on *P. foliosa*, *P. grandiflora*, *Talinum triangulare* or *Axonopus compressus*.

The eggs of *H. missipus* hatched within 3 to 4 days. There were four instars on *P. oleracea* and *P. quadrifida* but five instars on *A. gangetica* (Tables 1 and 2). Day-old larvae had a body length of about 0.15 cm and weighed less than 0.01 g. Males attained a length of 2.5-3.5 cm and a weight of 0.8-1.0 g while females were 3.0-4.0 cm long and weighed 1-1.2 g before pupation. Larvae that developed on *P. oleracea* produced the heaviest pupae (Table 3) and largest adults, with females having a mean wing span of 9.4 cm and body length of about 3.0 cm while males had a mean wing span of 7.8 cm and body length of 2.7 cm (Figs 1 and 2). Males developed relatively faster than females on all the food plants (Table 1), while female pupae were heavier than male pupae (Table 3).

When reared on *P. oleracea*, male larvae developed 3.5 days faster ($p<0.000$; $\chi^2 = 26.008$) and females 3.6 days faster ($p<0.000$; $\chi^2 = 28.468$) than those reared on *P. quadrifida*. Also, larval period was shorter on *P. quadrifida* than on *A. gangetica* by 6.1 days for males ($p<0.000$; $\chi^2 = 27.854$) and 7.6 days for females ($p<0.000$; $\chi^2 = 17.351$). However pupal periods were similar among all the food plants (male: $p=0.142$; female: $p=0.262$). Larval mortality was lowest on *P. oleracea* (8.7%), followed by *P. quadrifida* (11.1%) then highest on *A. gangetica* (27.1%).

P. oleracea had the highest moisture content and highest levels of almost all the essential nutrients measured. *P. quadrifida* had the most fiber. *Asystasia gangetica* had the highest percentage of crude fat (Table 4).

In the field, *H. missipus* laid eggs on *P. quadrifida* and *P. oleracea*, and larvae survived on both plant species. In the laboratory, however, eggs were laid only on *P. quadrifida* or on other plants when mixed with *P. quadrifida*. *Portulaca quadrifida* and *P. oleracea* may have similar chemical compounds that attracted *H. missipus* for oviposition in the field. However in the laboratory, *P. oleracea* could not attract the butterfly for oviposition. *Portulaca quadrifida* remains fresh for a long period of time and may continue to grow after it has been uprooted. That is not the case for *P. oleracea*, which dehydrates very quickly when out of the soil. Dehydration could cause the breakdown of attractants or the production of stress chemicals that did not attract butterflies. This may explain why in the laboratory, *P. quadrifida* was still able to attract the butterfly for oviposition but *P. oleracea* could not.

In the laboratory, while eggs were laid only on *P. quadrifida* when larval food plants were presented separately, eggs were also laid on other plants when those were mixed with *P. quadrifida*. It appears that ovipositing butterflies are unable to distinguish larval food plants mixed with other plants. This phenomenon, however, is not likely to affect the survival of the larvae in the field, because they are mobile and able to search for the appropriate food plant. Thus even when the eggs are laid on plants that the larvae will not feed on, the neonate larvae may be able to reach preferred food plants, particularly when food plants are not too distant from the oviposition site. This ability was evident in the laboratory when eggs were laid on *Axonopus compressus* that was mixed with *P. quadrifida*. The hatchling larvae did not eat the grass, nor is there any published record of it as a larval food of *H. missipus*. This study provides the first record of an egg laid on *A. compressus* or any grass. The larvae that emerged from eggs laid on *A. compressus* in the laboratory were able to locate and feed on *P. quadrifida* that had been placed in the same container. This behaviour could have survival value

Table 4. Nutrient content of *P. oleracea*, *P. quadrifida* and *A. gangetica*.

Food plant	Nutrient content (%)					
	Moisture	Crude protein	Crude fat	Fibre	Ash	Soluble carbohydrate
P. oleracea	92.5	26.7	11.5	11.2	22.1	15.1
P. quadrifida	88.6	11.4	8	14.1	11.2	6.6
A. gangetica	81.6	25	12.6	12.8	15.8	9.2

in nature, as eggs laid away from larval food might be protected from natural enemies that search for eggs on certain larval food plants.

In the laboratory, larvae developed at different rates on *P. oleracea*, *P. quadrifida* and *A. gangetica*. Nutrients influence all aspects of insect growth, development, and reproduction; *H. missipus* must obtain adequate amounts of the necessary nutrients in a suitable relative balance. Among the three food plants studied, larvae of *H. missipus* performed best on *P. oleracea* in terms of development time and adult size. There is no published report on the essential nutrient requirements of *H. missipus* larvae, but the good performance of larvae on *P. oleracea* indicates the presence of adequate amounts of the essential nutrients required by *H. missipus*. This assessment is supported by the plant nutrient content analysis, which showed that *P. oleracea* had the highest levels of almost all the important nutrients required for insect growth and development. Other studies have shown that *P. oleracea* also contains high levels of Omega-3 fatty acids, in particular alpha-linolenic acid (Simopoulos *et al.*, 1992), which is one of the major fatty acids in insect triglycerides and phospholipids, and is a dietary requirement for lepidopterans (Chapman, 1998). Deficiency of this polyunsaturated fatty acid in lepidopterans can cause failure of pupal or adult ecdysis (Nation, 2008).

The longer development period and smaller size of butterflies when reared on *A. gangetica* may be due to *A. gangetica* not having adequate amounts of the essential nutrients and water required by the larvae for optimum growth and development. Thus it took larvae longer to accumulate the amounts of nutrients necessary to reach pupation. It could also be that the plant did not stimulate the larvae enough to feed properly. Thus the larvae needed more time and an extra instar before they could pupate. Commonly, as food intake increases, development period is extended and insects become smaller and lighter in weight (Chapman, 1998). On nutritionally poor diets, low growth rates are associated with an increase in the number of larval stages. For instance, the caterpillars of *Spodoptera exempta* grew more slowly on *Panicum* and *Setaria* than they did on *Cynodon*, which is more nutritious (Yarro, 1985).

In other countries, including neighboring Côte d'Ivoire, *Talinum triangulare* is known to support development of *H. missipus* (unpublished observations; Vane-Wright *et al.*, 1977) but in our study, larvae did not survive on that plant. Perhaps *H. missipus* may be adapted to different food plants in different localities or geographical areas.

This study has shown that *H. missipus* can survive on *P. oleracea*, *P. quadrifida* and *A. gangetica* but that it develops more quickly and reaches larger size on *P. oleracea*. The information provided about the interactions between *H. missipus* and its food plants is important especially for conservation programs and the mass rearing of *H. missipus* either for research or ecotourism, when choice of appropriate food plants or oviposition materials is necessary. A thorough knowledge in this area is basic to development of an understanding of the butterfly's behaviour, biology and ecology as well as to the development of conservation strategies.

There is a need for further studies on the food requirements of *H. missipus* to fully explain the different larval growth rates recorded for different plants, in this study. Quantifying the amount of food eaten by the larvae on each food plant could demonstrate whether one food plant stimulates feeding better than the others.

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B. A. Mensah* and A. A. Kudom, Department of Entomology and Wildlife, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana.

**Corresponding author*
b2mensah@yahoo.com